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A diterpenoid sugiol from *Metasequoia glyptostroboides* with α-glucosidase and tyrosinase inhibitory potential

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Abstract

Now-a-days use of plant derived natural compounds have become a topic of increasing interest in food and medicine industries due to their multitude of biological and therapeutic properties. In this study, a diterpenoid compound sugiol, isolated from *Metasequoia glyptostroboides* was evaluated for α-glucosidase and tyrosinase inhibitory efficacy in terms of its potent anti-diabetic and anti-melanogenesis potential, respectively. As a result, sugiol at the concentration range of (100-10,000 µg/mL) and (20-500 µg/mL) showed potent efficacy on inhibiting α-glucosidase and tyrosinase enzymes *in vitro* ranging from 12.34-63.5 and 28.22-67.4%, respectively. These findings confirm the therapeutic potential of diterpenoid compound sugiol from *M. glyptostroboides* as a novel candidate for using in food and medicine industry which may have practical potential to cure skin and diabetes mellitus type-2 related disorders.

Introduction

The early stage of diabetes mellitus type 2 is associated with hyperglycemia due to impaired after-meal acute insulin secretion. Hyperglycemia is believed to increase the production of free radicals and reactive oxygen species, leading to oxidative tissue damage and diabetic complications (Maritim et al., 2003). Glucosidases are a group of digestive enzymes which break down the dietary carbohydrates into simple monosaccharides. Glucosidase inhibitors reduce the rate of carbohydrate digestion and delay the carbohydrate absorption from the digestive tract. Therefore, they have a potential to prevent the development of type 2 diabetes mellitus by lowering the after-meal glucose levels (Liu et al., 2011).

Tyrosinase, a copper-containing polyphenol oxidase, plays a highly critical role in forming melanin pigments (Ozer et al., 2007). Previous reports have shown that tyrosinase might also be involved in neuromelanin production and be associated with Parkinson's disease (Chen et al., 2013). Therefore, inhibiting tyrosinase activity is applicable to skin-lightening and in preventing neurodegeneration (Kwon et al., 2011).

Phytochemicals confer various health benefits, among them, α-glucosidase and tyrosinase inhibitory activities have particularly received intensive attention due to the increasing number of patients suffering from diabetes type 2 and skin disorders. Though synthetic α-glucosidase and tyrosinase inhibitors have been used effectively, many doubts have been raised on their safety such as increased toxicity and adverse side effects. Hence, attention has been focused on the effective use of plant based compounds which are less toxic and natural in origin.

Although the biological efficacy of *M. glyptostroboides* derived compounds has been reported previously (Bajpai et al., 2010; Bajpai et al., 2011; Bajpai et al., 2014),

there is no report available on α-glucosidase and tyrosinase inhibitory effects of sugiol, a diterpenoid isolated from *M. glyptostroboides*. Hence, the aim of this research is to confirm the therapeutic efficacy of sugiol as a potent α-glucosidase and tyrosinase inhibitor.

Materials and Methods

Chemicals and instrument

Kojic acid, acarbose, sodium azide (NaN_3), bovine serum albumin, p-nitrophenyl-α-D-glucopyranoside, yeast α-glucosidase, mushroom tyrosinase, and 3,4-dihydroxy-L-phenylalanine (DOPA) were purchased from (Sigma-Aldrich, USA). All other reagents used were of high analytical grade. Spectrophotometric measurements were done by using a 96-well micro-plate ELISA reader (Infinite M200, Switzerland).

Plant material

The cones of *M. glyptostroboides* were collected from Pohang city, Korea, and identified by the morphological features and the database present in the library at the Department of Biotechnology, Daegu University, Korea. A voucher specimen (DUB-0038) was deposited in the herbarium of College of Engineering, Department of Biotechnology, Daegu University, Korea.

Extraction and isolation of sugiol

Dried cones of *M. glyptostroboides* (2 kg) were milled into powder and then extracted with ethyl acetate at room temperature for 12 days. The extract was evaporated under reduced pressure using a rotary evaporator (EYELA N1000, Japan). The dried ethyl acetate extract (7 g) was subjected to column chromatography over silica gel (mesh 230-400 mesh, Merck, Germany) and was eluted with hexane-ethyl acetate-methanol solvent system to give 20 fractions. Of the fractions obtained, fraction-14 was further purified by preparative thin layer chromatography (TLC) over silica gel GF_{254} using hexane-ethyl acetate (2:1) as a mobile phase to give one compound (122 mg) which on the basis of spectral data analysis was characterized as sugiol shown in Figure 1 (Bajpai et al., 2011).

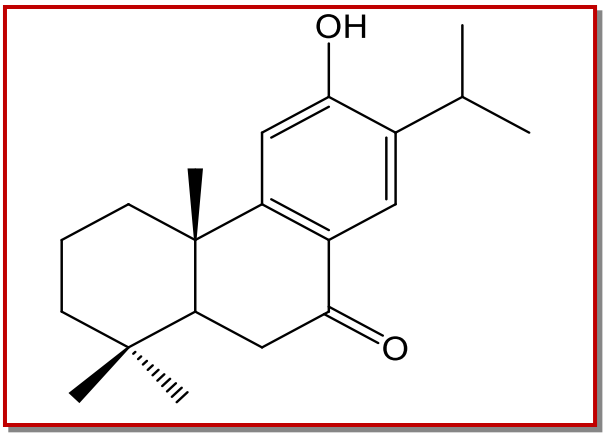

Figure 1: Chemical structure of a diterpenoid sugiol isolated from *Metasequoia glyptostroboides*

Assay of α-glucosidase inhibition

α-Glucosidase inhibitory activity of sugiol isolated from *M. glyptostroboides* was evaluated according to the chromogenic method (Liu et al., 2014). Briefly 10 µL of test samples at various concentrations (100, 500, 1,000, 5,000 and 10,000 µg/mL) and 50 µL of yeast α-glucosidase, dissolved in 100 mM phosphate buffer (pH 7.0) (containing 2 g/L bovine serum albumin and 0.2 g/L NaN_3) were mixed in 96 well micro-plate and absorbance at 405 nm was measured for titer at zero time with a micro-plate reader (Tecan, Infinite M200, Switzerland). After 5 min incubation, 50 µL of p-nitrophenyl-α-D-glucopyranoside (5 mM) in the same buffer (pH 7.0) was used as a substrate solution and incubated for an additional 5 min at room temperature. Eventually the reaction was terminated by adding 80 µL of 0.2 M sodium carbonate solution. Absorbance of the reaction mixture was measured with a micro-plate reader at 405 nm. The increase in absorbance from zero time was measured. Inhibitory activity was expressed as 100 minus relative absorbance difference (%) of test compounds to absorbance change of the control, while the reaction system without sugiol was served as a control test. The system without α-glucosidase was used as blank, and acarbose at various concentrations (100, 500, 1,000, 5,000 and 10,000 µg/mL) was used as a positive control. Each experiment was conducted in triplicate, and the enzyme inhibitory rate was calculated as follows:

Inhibition (%) = (Control absorption - Sample absorption)/Control absorption × 100

Assay of tyrosinase inhibition

The tyrosinase activity of sugiol was measured by a previously reported method (Fawole et al., 2012). Briefly, 100 µL of different concentrations (20, 40, 100, 200 and 500 µg/mL) of sugiol were mixed with 600 µL of 0.175 M sodium phosphate buffer (pH 6.8). Further, 200 µL of 10 mM L-DOPA solution (L-3,4-dihydroxyphenyl-alanine) was added to each well. After that, 200 µL of tyrosinase (110 units/mL in 0.175 M sodium phosphate buffer) was added to the reaction mixture and further incubated at 37°C for 2 min. Then after incubation, the amount of dopachrome produced in the reaction mixture was measured at 475 nm in a 96-well micro-titer plate with a micro-plate reader. Kojic acid (20, 40, 100, 200 and 500 µg/mL) was used as a positive control. The experiment was conducted in triplicate at room temperature, and the enzyme inhibitory rate was calculated as follows:

Inhibition (%) = (Control absorption - Sample absorption)/Control absorption × 100

Statistical analysis

All the data were expressed as mean ± standard deviation of three replicates. Tests of significant differences were determined by one-way ANOVA followed by Duncan's test using SAS software (SAS 9.2, SAS), and the values were considered to be significant ($p<0.05$).

Results and Discussion

Identification of sugiol

The ethyl acetate cone extract of *M. glyptostroboides* after column chromatography over silica gel yielded a pure compound, which was obtained as yellow glass crystal with a specific melting point (mp 282-84°C). The ^{1}H NMR data (pyridine-d5, 250 MHz) showing two singlet protons at d 8.39 and 7.16 (each 1H, s), an aliphatic methine signal at d 3.59 (1H, m, signals partially overlapped), and five terminal methyl groups at d 1.35 (3H, d, *J* = 6.8 Hz), 1.33 (3H, d, *J* = 6.8 Hz), 1.11 (3H, s, Me-20), 0.83 (3H, s, Me-19), 0.79 (3H, s,Me-18), as well as the ^{13}C NMR data displaying twenty carbon signals including a carbonyl group at d 197.6 strongly suggested that this compound should be an abietane diterpenoid. The structure of this compound was determined to be sugiol (Figure 1) by 1D and 2D NMR analysis, and confirmed by comparing the physical and spectroscopic data with those in the literature (Chang et al., 1990; Bajpai et al., 2011).

Inhibition of α-glucosidase

Phytochemicals have become important sources of human therapeutics and potential exists for their use in developing novel diabetes therapies or bio-therapeutic agents (Tiwari et al., 2010). There is a huge ongoing interest in the application of plant-based compounds and/or products for alleviating chronic diseases due to their potent therapeutic and medicinal efficacy. Still the use of phytochemicals within the context of diabetes remains largely unexplor-ed; which has urged the scientists to provide scientific evidences on the development of more effective agents conferring inhibitory effects on intestinal glucosidases. As reported previously, to control hyperglycemia, inhibition of intestinal α-glucosidase is an established strategy (Tiwari et al., 2010; Ma et al., 2014). Although there have been reports on the clinical availability of α-glucosidase inhibitors, there is still need to develop alternative therapies to inhibit this key enzyme in order to minimize side effects and drug cost efficacy. In this regard, several natural substances from plant origins have been analyzed for their enzymatic inhibitory activities (Tiwari et al., 2010; Ma et al., 2014; Liu et al., 2014).

The α-glucosidase inhibitory activity of sugiol was found to be in a concentration dependent manner. The inhibitory effect of sugiol on α-glucosidase has been demonstrated in Figure 2. The sugiol at various concentrations (100, 500, 1,000, 5,000 and 10,000 µg/mL) showed the inhibition of α-glucosidase by 12.3, 24.5, 32.2, 51.3 and 63.5%, respectively. In case of standard

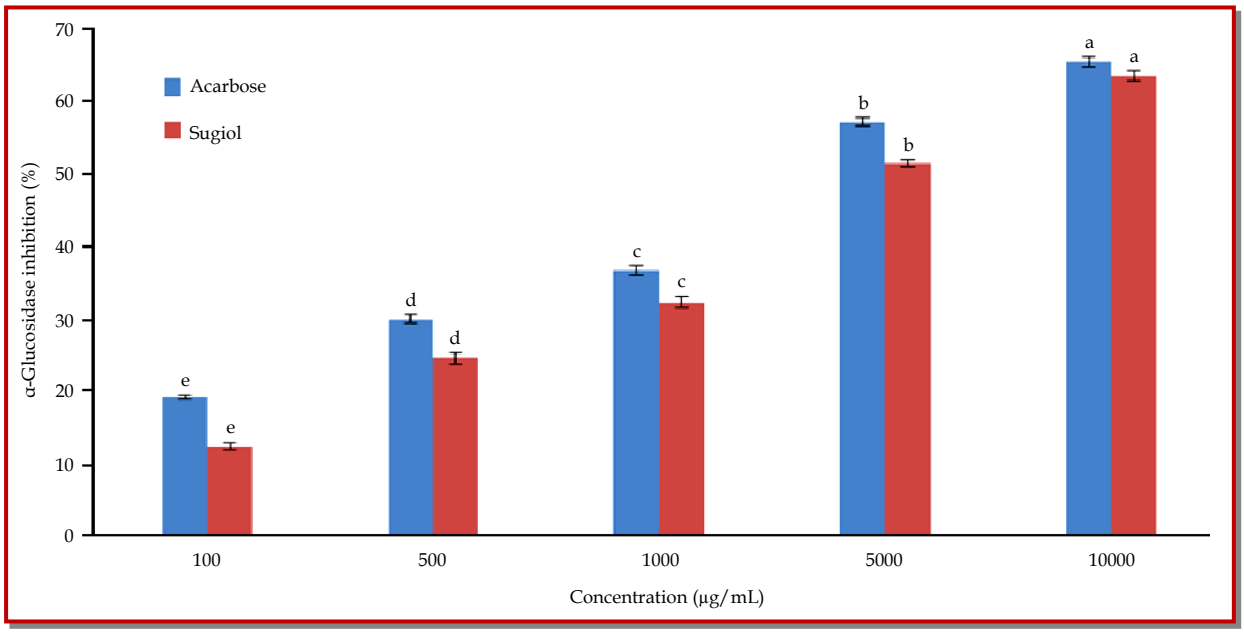

Figure 2: α-Glucosidase inhibitory effect of standard compound acarbose and sugiol isolated from *Metasequoia glyptostroboides*.

Data are expressed as mean ± SD (n = 3). Values with different superscripts are significantly different ($p<0.05$)

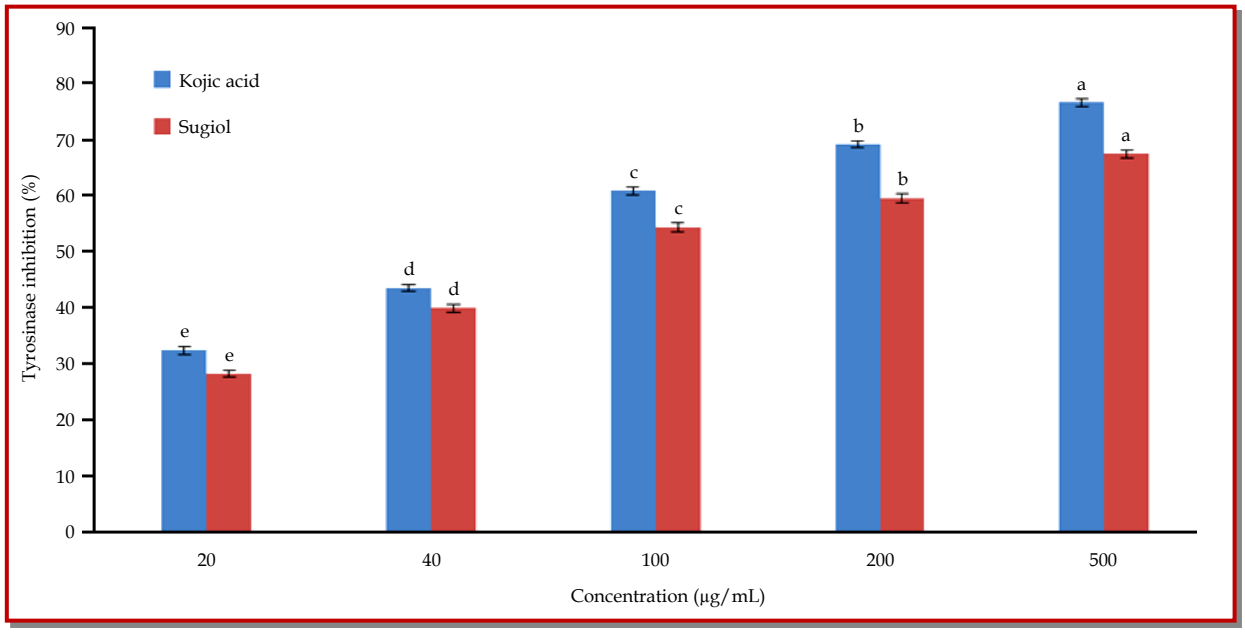

Figure 3: Tyrosinase inhibitory effect of standard compound kojic acid and sugiol isolated from *Metasequoia glyptostroboides*. Data are expressed as mean ± SD (n = 3). Values with different superscripts are significantly different ($p<0.05$)

acarbose at 100, 500, 1,000, 5,000 and 10,000 µg/mL, it displayed α-glucosidase inhibitory activity by 19.2, 29.9, 36.7, 57.1 and 65.5%, respectively. In this assay, the test compound sugiol showed inhibitory effect in a dose-dependent manner as did by the standard compound acarbose. Similar findings on α-glucosidase inhibitory activity of flavonoid and terpenoid compounds isolated from *Agrimonia pilosa* were observed by Liu et al. (2014). In addition, sarcoviolins isolated from edible mushroom *Sarcodon leucopus* were also found to inhibit α–glucosidase *in vitro* (Ma et al., 2014). Currently α-glucosidase inhibitors are used for the treatment of type 2 diabetes mellitus, suggesting that sugiol in formulation might be used as an oral hypoglycemic agent.

Inhibition of tyrosinase

Melanin is a polymerized natural coloring determinant in animal, plant, and microorganisms. A number of melanins are produced by multi-stepped enzymatic and non-enzymatic oxidation and polymerization processes. In mammals, the melanin can be divided into eumelanin and pheomelanin due to its solubility in an alkali solution and the color reaction (An et al., 2005). The mechanism of tyrosinase inhibition activity may be an important factor in the skin whitening of a cosmetic composition (An et al., 2005). Melanin biosynthesis steps in the body include L-DOPA by tyrosine as a substrate, followed by its conversion to L-dopaquinone by the successive enzymatic oxidations. Finally, a polymerization reaction takes place (An et al., 2005).

The inhibitiory effect of diterpenoid compound sugiol on the tyrosinase using a mushroom tyrosinase is demonstrated in Figure 3. In this assay, the sugiol at various concentrations (20, 40, 100, 200 and 500 µg/mL) showed 28.2, 39.9, 54.3, 59.5 and 67.4% inhibition of tyrosinase, respectively. Meanwhile, the mushroom tyrosinase inhibitory activity of standard compound kojic acid (20, 40, 100, 200 and 500 µg/mL) were found to be 32.4, 43.4, 60.8, 69.1 and 76.5%, respectively. Batubara et al. (2010) observed that the phyto-constituent taxifolin and some other flavanonol rhamnosides from *K. malaccensis* also suppressed tyrosinase activity in the range of 5.86-25.95%. Piao et al. (2002) demonstrated tyrosinase inhibitory effect of some chromones derived from a tropical plant aloe in a dose-dependent manner. In addition, the terpenoids isolated from the leaves of *Chloranthus tianmushanensis* were also found to exhibit tyrosinase inhibitor effect dose-dependently (Wu et al., 2008). Similar findings on dose-dependent tyrosinase activity of sugiol were observed in our study. Since it has been shown that tyrosinase inhibitors can repress the conversion of tyrosine to DOPA, dopaquinone and subsequently melanin, various tyrosinase inhibitors have been isolated and studied as potential candidates to decrease melanin content. Our results suggest that sugiol derived from *M. glyptostroboides* might be used as a potential treatment of melanin-related disorders to serve as skin-whitening agent (Chai et al., 2012).

In this research work, a diterpenoid compound sugiol isolated from *M. glyptostroboides* demonstrated a considerable amount of α-glucosidase and tyrosinase inhibitory effects *in vitro* which may have potential to reduce blood glucose responses and to maintain skill health by inhibiting melanogenesis. The potent α-glucosidase and tyrosinase inhibitory efficacy of sugiol

in terms of anti-diabetic and skin-whiting efficacy makes it to be a molecule of choice for using in health-care and drug therapies in the treatment of infectious diseases. However, further studies are needed to demonstrate a precise mode of action of sugiol to confirm its *in vivo* anti-diabetic and anti-melanin potential.

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